Comparative Study for the Accuracy of *Helicobacter pylori* Diagnostic Methods Associated with Some Inflammatory Factors

**Eman N. Naji**
Department of Biology, College of Science, Mustansiriya University, IRAQ
Email: emannatiq@yahoo.com

**Abstract**
This Study was intended to diagnose *H. pylori* the major causative pathogen in gastro duodenal irritation and ulceration. Differert techniques were used invasive tests (histopathological examination, rapid urea CLO test and culture), while noninvasive tests includes (serological tests and stool antigen) in addition to determination of some immune response factors (IgM, IgG, IgA) as well as (IL – 8 and IFN – γ) in Ptients Sera.

According to the results of invasive diagnostic method 30/113 (26.69%) patients were considered to be infected and 83/113(73.31%) patients were considered as noninfeted was contrasted with noninvasive diagnostic method 25/113 (22.14%) patients were considered to be infected and 88/133(77.83%) patients were considered as noninfeted. In order to get the overall percentage of the infected people included in this study, we merge the results of the two methods, so we found out that the total infected patients with *H. pylori* diagnosed by invasive and noninvasive methods were 42 /113 (37.2%) while the noninfected 71/113 (62.8) disseminated as 27/68(39.71%) infected male, which was privileged than the infected female when it was 15/45(33.33%). The high prevalence of *H. pylori* infection in the age group ranging between (46-60) in male and female.

Histology (invasive teq.) and ECO rapid test (noninvasive teq.) were considered as the “best techniques” for *H. pylori* detection, in the outlook of its high specificity, sensitivity and because it detected the major number of *H. pylori*-positive patients along with the other techniques used in this work.

The sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) for histology were 100, 100, 100 and 94.5%, respectively, while for the ECO rapid test they were 96, 93, 91.5, and 97.14%. Culture (invasive teq.) and IgG anti *H. pylori* (noninvasive teq.) coming secondly in the diagnosis of *H. pylori* infection because they detected a little fewer number of infected patients than the first two teq. as noted above. The sensitivity, specificity, (PPV) (NPV) for Culture were 80%, 97%, 96.96% and 87.5% and for IgG anti *H. pylori* were 85%, 91%, 92.8% and 97.8%. Finally, the smallest patient number was obtained from the rest of all the six teq. were used in the present work obtained from the urea CLO test and stool antigen, invasive and noninvasive teq., respectively.

The present research found out that there were a relationship between the results of rapid anti *H. pylori* ECO test, antibody titer in ELFA, immunoglobulin (IgG and IgA) and (IFN-γ), (IL-8) concentration. Also, all these data were related to the results of histological changes and the results of the urea CLO test of patients when compared with the noninfected members. These results showed highly significant differences among patient groups in comparison with noninfected group at (P. Value < 0.001). On the other hand, there were no relationship between IgM concentration with any of the other results of diagnostic methods were used may such results considered a first step for determining the susceptibility of infection and to confirm the diagnosis by use one more test in each time especially Histology (invasive teq.) and ECO rapid test (noninvasive teq.) correlated with estimation of (IgG and IgA) and (IFN-γ), (IL-8) concentration.

**Keywords**: *Helicobacter pylori*, Gastric ulcer, Invasive test, noninvasive test, immunological parameters.

**Research Article**
Introduction

*Helicobacter pylori* is a Gram-negative, microaerophilic, and small corkscrew-shaped rod, extremely motile bacterium that colonizes just in the mucous layer of the human stomach, is an essential pathogenic factor in chronic energetic gastritis, duodenal and gastric ulcers,[1,2] affects more than semi of human population international and is mainly more settled in developing countries[3,4].

*H. pylori* in extraordinary in its ability to colonize the stomach, where low pH normally protects against bacterial infection. This bacterium colonizes gastric mucosal cells in the stomach, surviving in the mucous layer that coats the epithelium. The organism is noninvasive, but recruits and activates inflammatory cells, thus causing a chronic inflammation of the mucosa.

*(H. pylori)* secrete urease, producing ammonium ions that neutralize stomach acid in the vicinity of the organism, thus favoring bacterial multiplication.[5]

There are now several invasive methods for the clinical diagnosis of *H. pylori*, such as histopathology examination (HE), rapid urea (CLO) test, and bacterial culture as well as noninvasive methods such as serology, 13C-urea breath test, and the stool antigen test[6,7]. Regardless of the fact that several invasive and noninvasive methods exist for the diagnosis of *H. pylori*, none of these have been conventional as a gold standard[9,10]. Infection with this organism induces inhibition of polymorphonuclear and mononuclear leukocytes and enhances the creation of various cytokines in gastric mucosa [9,10]. This development enhances protein secretion of interleukin (IL) -8 and interferon-γ (INF-γ) production they can be detected in serum of *H. pylori* patients assumed to have infection can be used to find out the incidence of both acute and chronic infections. [15].

The earlier description of the significance and occurrence diagnostic methods of *H. pylori* infections associated with the determination of some inflammatory factors this study aimed to diagnose *H. pylori* in patients assumed to have gastric ulcers by both invasive methods includes histopathological examination (HE), rapid urea (CLO) test and culture in addition to noninvasive tests includes serological tests and stool antigen test, or else determine some humoral immune response factors (IgM, IgG, IgA), and detect the (IL-8 and IFN-γ) in patient sera.
Materials and Methods

Patients and Sample collection: The specimens were collected under physician medicine conference during the period between April 2015 and December 2016 from different private clinics and hospitals in Baghdad. One hindered thirteen volunteers consisted of 43 males and 70 females undergoing upper gastroduodenal endoscopy. These patients were admitted to the endoscopy unit of the Gastroenterology division. The patient consisted of participants that satisfied the following criteria: misery from pain with the itchy burning feeling; not having taken *H. Pylori* eradication treatment, antibiotic, or other drug within the last two weeks; and without bleeding and clotting disorders. The patient group was formed from patients in whom biopsy samples were found positive by at least two invasive diagnostic tests, such as histopathology and rapid urease test and/or bacterial culture. The noninfected group was formed of subjects whose biopsy samples were found negative for *H. pylori* by histopathology and rapid urease test and/or culture or if they were found positive by at least two non-invasive method used in this study while the others represent the noninfected group.

Gastric biopsies: biopsies were immediately separated into two portions one of them fixed at 10% buffered formalin to be used for the histopathological examination; the other part was ground at 10.000 rpm for 15 sec with an electric tissue homogenizer. The homogenized tissue separated in to two portions one of them used in rapid urea (CLO) test at the same time as the other parts immediately placed in transport medium in order to use in a bacterial culture.

Blood sample:

Five mL of blood was collected in dry tubs without anticoagulant, after clotting, the sera were obtained by centrifugation (for 10 min at 5000 rpm) divided into aliquots and stored at (-20°C) until used in the serological and immunological test.

Stool samples:

One to two grams of stool sample were collected in a dry cup in order to use it in the stool antigen test.

*H. pylori* infection diagnostic tests:

**Invasive test:** Histopathological examination (HE): For routine histology paraffin embedded tissue blocks were prepared and 5um thickness sections were mounted on slides for Hematoxylin and Eosin staining. Mucosal ulceration with heavy acute or chronic inflammatory cells infiltrate were detected. Giemsa stain was used to search for bacteria within the tissue [11].

Culture (bacterial isolation): The biopsy portion was put in transport media immediately cultured on Colombia agar plates containing 5% defibrinated sheep blood, 10 mg/L Vancomycin, and 5 mg/L Trimethroprim and incubated in 5% CO2 incubator (microaerobic conditions) for 3-5 days. Organisms were identified as *H. pylori* by colony morphology of bacteria and their Gram-staining characteristics were studied. Convex semitransparent, 1-2 mm diameter colonies with the positive reaction of catalase, urease, and oxidase [15].

**Rapid urea (CLO) test:**

The CLO test rapid urease test (Kimberly-Clark/U. S. A) is a variation of the test where the biopsy sample is placed in a medium containing urea.

A marker is then used to determine if a chemical reaction has taken place to suggest the presence of the *H pylori* bacterium. This reaction takes place (10 min-24 hour) the areas of *H pylori* hydrolyze urea to release ammonia, which is detected colorimetrically and can be used as a diagnosis of an infestation by the *H. pylori* bacterium. When the CLO test is positive (red color reaction) it is a fairly reliable indicator that the individual is suffering from an infection of this bacterium, while the negative reaction (yellow color reaction) it means that the individual is not infected.

**Noninvasive Tests**

**Rapid Anti *H. pylori* Test:**

*H. pylori* antibodies Rapid Test Device (serum /plasma) was used as a rapid visual immunoassay for the qualitative presumptive detection of specific IgM and IgG antibodies to *H. pylori* in human serum specimens. The procedure was done according to manufacture instructions (ECOtest D-HP-32). The device and the specimens were
brought to room temperature and 75μl from the serum was transferred to the specimen well. Migration of specimen across the resort area in the center of the device will cause coloration (dark red color) of control band and another red band appeared within five minutes in case of a positive result, while the only red control band appears in the negative results. Invalid: There should always be a purplish red control band in the control region regardless of test results. If a control band is not seen, the test is considered invalid. Deep of the color and time of result appearance was recorded.

**Quantitative determination of IgG-class antibodies against H. pylori by Enzyme Linked Fluorescent Assay (ELFA)**
The Vidas is an automated qualitative test for use of the instruments of the Vidas family. For the detection of anti-Helicobacter pylori IgG antibodies in human serum or plasma using the ELFA technique. The procedure was done according to manufacture instructions of IgG-class antibodies kit (Biomerieux, France).

**Faecal antigen test:**
The *H. pylori* stool antigen test was performed to detect the presence of *H. pylori* infection in the patient and control groups. Stool samples were analyzed using the ABON H. pylori antigen test device (Abon Biopharm, Germany), that is, a lateral flow chromatographic immunoassay for detection of *H. pylori* antigen. A diluted stool sample was dispensed into the sample port of the test device, and the appearance of a colored line after 10 min in the test line region of the strip indicated a positive result.

**Determine some humoral immune response factors:**
Determination of Human Immunoglobulinse (IgM, IgG and IgA) Turbidimetry method. This method depends on the quantitative determination of human Immunoglobulins IgG, IgA, IgM without sample dilution. The procedure was done according to manufacture an instructions kit (Human, Germany).

**Determination of Human Interferon Gamma (IFN-γ) Interferon Gama (IFN-γ)** according to the protocol of Human IFN-γ ELISA (Enzyme-Linked Immunosorbent Assay) kit was used for the quantitative measurement of human IFN-γ in serum.

**Determination of human (IL-8)**
Interlukin-8 (IL-8) estimated according to the protocol of Human (IL-8) ELISA Kit. The IL-8 EASIA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiterplate.

**Statistical Analysis**
Statistical analysis was performed using SPSS v20.0 software (SPSS Inc, Chicago, IL, USA). Differences were considered significant when p ≤ .05.

**Results and Discussion**

**Subject analysis**
One hundred and thirteen people's 68 male (60.18%) age ranges between 30-72 years while median age was 51 and 45 females (39.82%) age ranges between 32-70 years while median age was 48.5 years. Patients were divided into three age groups as listed in the Table 1. All patients were subjected to gastroendoscopy, venous blood and stool samples were collected from patients for diagnostic methods and some immunological tests used in this study.

**Determination of *H. pylori* in Patients by invasive and noninvasive diagnostic methods:**
Methods that exactly detect *H. pylori* infection in dyspeptic patients are major importance. Direct manifestation of *H. pylori* in gastric biopsy specimens is possible through the use of histological examination with Giemsa staining, culture, and assays for rapid urea (CLO) test. All these endoscopy-based methods require gastric biopsy specimens and are thus classified as invasive methods (7).

<table>
<thead>
<tr>
<th>Gender</th>
<th>No 113 (%)</th>
<th>Age /year</th>
<th>Age groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male No.68 (60.18)</td>
<td>30-72</td>
<td>30-45</td>
<td>20</td>
</tr>
<tr>
<td>Female No.45 (39.82)</td>
<td>32-75</td>
<td>48.5</td>
<td>16</td>
</tr>
</tbody>
</table>
H pylori infection elicits a local mucosal and a systemic antibody response, circulating IgG antibodies to H pylori can be detected by Enzyme Linked Fluorescent Assay (ELFA) antibody, Rapid anti H pylori ECO20E test and other serological test, these two tests and detect the presence of H pylori antigens shed in the faeces involved in the noninvasive methods were used in this study. The patient group was selected from patients in whom biopsy samples were found positive by at least of two invasive diagnostic tests, and/or if they were found positive by at least of two noninvasive methods used in this study while the others represent the noninfected group (16).

Results of invasive test:
In this study, the presence of H pylori was resolve by invasive techniques (histology, rapid urea CLO test and culture) of gastric antral biopsy specimens in 113 suspected patients. As shown in table (2) and Figure (1A and B), 17 (15.15%) patients were positive in the three tests, 11 (9.76%) patients were positive in both culture and rapid urea CLO test, 2 (1.78%) patients were positive in both histological examination and culture, otherwise 3 (2.56%) patients were found to be positive only in histological examination and, while 2 (1.78%) patients were positive in a bacterial culture. Patients were considered to be infected with H pylori if they were positive in two of the three tests as we noted. So, according to these results of invasive diagnostic method 30/113 (26.69%) patients were considered to be infected and 83/113 (73.31%) patients were considered as noninfected.

<table>
<thead>
<tr>
<th>Invasive methods</th>
<th>Histology</th>
<th>Culture</th>
<th>CLO test</th>
<th>No. (113)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>17</td>
<td>15.15*</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td>2</td>
<td>1.78*</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td>3</td>
<td>2.65</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td>11</td>
<td>9.76*</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td>1</td>
<td>0.88</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
<td>2</td>
<td>1.78</td>
</tr>
</tbody>
</table>

*Infected groups

Figure 1: A. The results of 1: urea CLOtest, 2: positive culture of H pylori and 3: Gram stains reaction.
Results of noninvasive test:
In order to confirm the results we have obtained in determination of *H. pylori* infected patients by invasive method we were undertaking a three of noninvasive techniques as shown in Table 3 Figure 2, 19 (16.9%) patients were only positive in ECO test, 11 (9.76%) were positive in both IgG against *H. pylori* and ECO test, otherwise 13 (11.5%) patients were found to be positive in three noninvasive tests and 1 (0.88%) patients were positive in both IgG against *H. pylori* and fecal antigen test, while 1 (0.88) patients were positive in a Fecal antigen test. Patients were considered to be infected with *H. pylori* if they were positive in two of the three tests as we noted. So, according to these results of noninvasive diagnostic method 25/113 (22.14%) patients were considered to be infected and 88/133 (77.83%) patients were considered as non-infected.

<table>
<thead>
<tr>
<th>(%)</th>
<th>NO (113)</th>
<th>Fecal antigen test</th>
<th>IgG against <em>H. pylori</em></th>
<th>Rapid anti <em>H. pylori</em> ECO test</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.5*</td>
<td>13</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9.76*</td>
<td>11</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>16.9</td>
<td>19</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 3: Results of endurance of *H. pylori* in Patients by noninvasive methods.
Invasive and noninvasive tests can be used and recommended to correctness for the in vitro diagnosis of *H. pylori*, which has a role in the pathologies of gastritis [17].

In order to get the overall percentage of the infected people included in this study, we merge the results of the two methods (inv. and noninv.) as listed in Table (4) and Figure (3), where it was split percentage and the number of infected patients associated with sex and three age groups. The results showed that the total infected patients with *H. pylori* diagnosed by invasive and noninvasive methods were 42/113 (37.2%) while the noninfected 71/113 (62.8) disseminated as 27/68 (39.71%) infected male, which was privileged than the infected female when it was 15/45 (33.33%). The high prevalence of *H. pylori* infection in the age group ranging between 46-60 in male and female were 15/68 (35.71%) and 9/45 (21.43%) respectively, there was highly statistically significant differences at p value (0.02).

Our results explained that there is a relation between the age and the incidence of *H. pylori*. The results of the current work disagree with the results of [18] who found that there is no significant difference in *H. pylori* prevalence among patients have the same age range and a different gender, and agree with the study of [19] which showed a very high incidence of *H. pylori* infection in the age group ranging from 41-50 and 51-60 years. The differences among the results might due to some factors such as skin and blood classification, habitates, teaching level and smoking [20].
Table 4: Number and presentages of infected patients with *H. pylori* associated with age groups and gender.

<table>
<thead>
<tr>
<th>PV</th>
<th>Female NO:45</th>
<th>Male NO:68</th>
<th>Gender Age groups/Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05 (S)</td>
<td>Negative NO (%) 10(22.23)</td>
<td>Positive NO (%) 5(11.11)</td>
<td>30-45 (No. : 31), M:16 // F:15</td>
</tr>
<tr>
<td>0.02 (S)</td>
<td>Negative NO (%) 15(33.33)</td>
<td>Positive NO (%) 9(20)</td>
<td>46-60 (No. :58), M:34 // F:24</td>
</tr>
<tr>
<td>0.01(S)</td>
<td>Negative NO (%) 5(11.11)</td>
<td>Positive NO (%) 1(2.2)</td>
<td>61-72 (No. :24), M:18 // F:6</td>
</tr>
<tr>
<td>0.01(S)</td>
<td>Negative NO (%) 30(66.67)</td>
<td>Positive NO (%) 15(33.33)</td>
<td>Total</td>
</tr>
</tbody>
</table>

Figure 3: The distribution of patients' number infected with *H. pylori* associated with age groups and gender.

The relative results obtained by all the diverse tests used in the current study are listed in table (5), showed that histology (invasive teq.) and ECO rapid test (noninvasive teq.) were considered as the “best techniques” for *H. pylori* detection, in the outlook of its high specificity, sensitivity and because it detected the major number of *H. pylori*-positive patients along with the other techniques used in this work. The sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) for histology were 100, 100, 100 and 94.5%, respectively, while for the ECO rapid test they were 96, 93, 91.5, and 97.14%.

Culture (invasive teq.) and IgG anti H. pylori (noninvasive teq.) coming secondly in the diagnosis of *H. pylori* infection because they detected a little fewer number of infected patients than the first two teq. as noted above. The sensitivity, specificity, (PPV) (NPV) for Culture was 80%, 97%, 96.96% and 87.5% and for IgG anti H. pylori were 85%, 91%, 92.8% and 97.8%. Finally the smallest patient number was obtained from the rest of all the six teq. were used in the present work they were the urea CLO test and stool antigen, invasive and noninvasive teq. respectively. The sensitivity, specificity, (PPV) (NPV) for these tests was explained in Table 5.
Table 5: The relative accuracy of invasive and non invasive tests for *H pylori* infection.

<table>
<thead>
<tr>
<th>Test type</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>*PPV %</th>
<th>^NPV %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Invasive test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td>100 %</td>
<td>100 %</td>
<td>100%</td>
<td>94.5%</td>
</tr>
<tr>
<td>Culture</td>
<td>80 %</td>
<td>97 %</td>
<td>96.96%</td>
<td>87.5%</td>
</tr>
<tr>
<td>Urea CLO test</td>
<td>91 %</td>
<td>89 %</td>
<td>85.71%</td>
<td>98.7%</td>
</tr>
<tr>
<td><strong>Noninvasive test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapid ECO test</td>
<td>95 %</td>
<td>94 %</td>
<td>91.5%</td>
<td>97.14%</td>
</tr>
<tr>
<td>IgG anti <em>H. pylori</em></td>
<td>85 %</td>
<td>91 %</td>
<td>92.8%</td>
<td>97.6%</td>
</tr>
<tr>
<td>Stool antigen</td>
<td>83 %</td>
<td>89 %</td>
<td>78.9%</td>
<td>96.9%</td>
</tr>
</tbody>
</table>

PPV: Positive predictive value, means that if the test positive, you have a (according to the test type) % chance of actually having the disease.

NPV: Negative predictive value, means that if the test negative, you have a according to the test type % chance of not having the disease.

In the present study, six techniques were used to detect infection of *H. pylori* in random Iraqi people, including invasive and noninvasive technique. Numerous people get *H. pylori* through childhood, but adults can get it from food and drinks or by get in touch with the saliva or body fluids of infected people. It’s further frequent in countries that suffering from contaminated water with sewage [20]. In a study carried by [21], they said that there is no particular test can be considered as the gold standard for the diagnosis of *H. pylori* infection and each technique has its private compensation and discompensation. That is depends on the decreasing sensitivity of each method. The isolation of the bacteria from gastric tissues by culture is difficult because of its low sensitivity. The critical troubles In the culture method such as the incubation conditions, media preparing, contamination problems, the slowly reproduction of the bacteria, strain type, technical difficulties and low diagnostic sensitivity of the method (80 % in the current study) , so it has not been used in the routine diagnosis, this result agreed with [23]. Gastric endoscopy is one of the important tests for *H. pylori* diagnoses neither by endoscopy examination and the diagnosis of patients' status or by taking the biopsy that it will be used in the all invasive diagnostic methods used for *H. pylori* infection [24].

In this study, we considered histology on of the “best techniques” for *H. pylori* detection, in the outlook of its high specificity, sensitivity and because it detected the major number of *H. pylori*-positive patients along with the other techniques used in this work,during histological examination detects lower stage of *H pylori* infection and this bacteria can be found in some sections stained with haematoxylin and eosin, some biopsy shows mild ulceration, atrophy of mucosal tissue, necrosis and infiltration.

The detection of tissue morphological changes because of *H pylori* infection is an important advantage of histology, in addition to the historical record provided, gastric or duodenal sections from biopsies (or even other sections) can be examined at any time[18] [25].

The urea CLO test and low expensive ureas tests are of comparable sensitivity and specificity. This simple tests used for detecting *H pylori* infection but indicate only the presence or absence of infection. Conversely, in this study the sensitivity of urease tests is frequently higher than that of culture (biopsy based technique) because the intact biopsy sample is placed in the media [26]. The CLO test, can consequence in fake positives for numerous reasons, contagion by other bacteria producing urease enzyme, mistaken completion of the CLO test during endoscopy, provisional reduce of bacteria due to antibiotics. As a result, when used alone, this test has low diagnostic concert [27].

For serological identification, rapid ECO test and ELFA are an uncomplicated, inexpensive more modern, successful method and because of their high specificity, sensitivity among other noninvasive test as listed in the Table 4 and can be made on frozined samples in addition this technique available in the private and public laboratories in Iraq. By using fecal antigen test there were no significant association was found.
between *H. pylori* stool antigen positivity and the other diagnostic methods were used in this study. However, stool antigen test can be used for diagnosis infection, specifically in children as the easy obtaining of stool sample and the most difficult to make endoscopy [28].

3- Results of Estimation of Immunoglobulins (IgM, IgG, IgA) and Interferon Gamma (IFN-γ) and Interleukin-8 (IL-8).

Estimation of immunoglobulins (IgG, IgA, IgM), Interferon Gamma (IFN-γ) and Interleukin-8 (IL-8) afford useful information for the assessment of convincing disease status. As revealed in the Table 6 the IgG, IgA, titers showed high concentration compared with the noninfected groups, the statistical analysis showed that there are significant differences at p value (0.001), while there were no statistically differences in IgM titer between the two tested group Table 6. [18] reported an important increase of IgG and IgA titer in *H. pylori* patients’ serum, but IgM does not present a further role. While [29] found that IgM has been create to have slight diagnostic efficacy for *H. pylori* infections and is superior only intensely following infection, whereas *H. pylori* infections are common chronic, that is IgM has exceptionally low sensitivity. Concentration of various cytokines, as well as interferon gamma (IFN-γ) and IL-8, are increased in the stomachs of *H. pylori*-infected patients compared to noninfected.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Immunoglobulins levels in serum (mg/dl)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>IgM</td>
<td>IgA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Infected (42)</td>
<td>1089.54±113.73</td>
<td>78.5±9.3</td>
<td>276.2±19.4</td>
<td></td>
</tr>
<tr>
<td>Noninfected (71)</td>
<td>467.88±79.53</td>
<td>63.4±7.98</td>
<td>122±11.8</td>
<td></td>
</tr>
<tr>
<td>P Value</td>
<td>0.001</td>
<td>NS</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

The cytokines titers showed that highly significant elevation of both cytokines (INF-γ and IL-8) among patient groups in comparison with noninfected group at (P. Value < 0.001) in Mean ± SD (140.40 ±61.08 and 241.72 ± 32.80 respectively) with (53.82 ±11.49 and 118.69 ±29.36 respectively) as a result mentioned in the table (7). The study of [18,29] showed that *H. pylori* induced considerably higher concentration of IFN-γ and IL-8, IFN-γ keeps mucosal inflammation and may encourage disease development to gastric ulcer. Another study [30], showed an increased concentration of IFN-γ in the stomachs of *H. pylori*-infected patients is dependable with the expansion of a Th1- largest response and another study by [31] reported that IL-8 is increased within *H. pylori*-infected mucosa where it localizes to gastric epithelial cells, and levels of IL-8 are directly associated to the strictness of gastritis as well IFN-γ, IL-8 increased values coincided with increased inflammation and with increased *H. pylori* density in humans [32] in addition to in animal model studies [33].

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Table 7: Statistical analysis of Interferon Gamma (IFN-γ) and Interleukin-8 (IL-8) concentration in *H. Pylori* infected and noninfected group.

<table>
<thead>
<tr>
<th>Study groups</th>
<th>INF-γ pg/ml, Mean ± SD</th>
<th>IL-8 pg/ml, Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected (42)</td>
<td>254.63±17.8</td>
<td>2.82±0.32</td>
</tr>
<tr>
<td>Noninfected (71)</td>
<td>23.76±1.2</td>
<td>0.26±0.04</td>
</tr>
<tr>
<td>P Value</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

The present results found out that there was a relationship between the results of rapid anti *H. pylori* ECO test, antibody titer in ELFA, immunoglobulin (IgG and IgA) and (IFN-γ), (IL-8) concentration. Also, all these data were related to the results of the histological changes and the results of the urea CLO test of patients when compared with the noninfected members. Such results could be considered the first step for determining the susceptibility of infection and to confirm the diagnosis by using one more test in each time. On the other hand, there was no relationship between IgM concentration with any of the other results of diagnostic methods used in our study, may be because of IgM antibodies against *H pylori* decrease with older age patients, which, since this is frequently asymptomatic, makes it difficult to identify cases of primary infection [34].

The majority of research merge two methods or further to get a magnificent diagnosis, including invasive or noninvasive methods and/or molecular method to advance diagnosis of *H. pylori* infection [35]. *H. pylori* in extraordinary in its ability to colonize the stomach and adhere to the epithelial cells by producing adhesions and causing gastric and peptic ulceration and other unusual changes, where low pH normally protects against bacterial infection[5]. For the reason that of the severe complicatedness that escort *H. pylori* infection which might have awful penalty, it is essential to clutch an early diagnosis to pass up the progress of the infection. Many particular methods had been used [36] or newly developed molecular techniques like Multiplex PCR was used for amplification the CagA genes assay to identify *H. pylori* in gastric biopsies [16] [36].

Our results indicate that there was a relationship between the results of rapid anti *H. pylori* ECO test, antibody titer in ELFA, immunoglobulin (IgG and IgA) and (IFN-γ), (IL-8) concentration. Also, all these data were related to the results of the histological changes and the results of the urea CLO test of patients when compared with the noninfected members. This result showed highly significant differences among patients groups in comparison with noninfected group at (P. Value < 0.001). On the other hand, there were no relationship between IgM concentration with any of the other results of diagnostic methods were used. Such results could be considered a first step for determining the susceptibility of infection and to confirm the diagnosis by using one more test in each time especially Histology (invasive teq.) and ECO rapid test(noninvasive teq.) correlated with estimation of (IgG and IgA) and (IFN-γ), (IL-8) concentration.

References


